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Review

Dysfunction of amyloid precursor protein signaling in neurons leads to DNA synthesis and apoptosis

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Abstract

The classic neuropathological diagnostic markers for AD are amyloid plaques and neurofibrillary tangles, but their role in the etiology and progression of the disease remains incompletely defined. Research over the last decade has revealed that cell cycle abnormalities also represent a major neuropathological feature of AD. These abnormalities appear very early in the disease process, prior to the appearance of plaques and tangles; and it has been suggested that neuronal cell cycle regulatory failure may be a significant component of the pathogenesis of AD. The amyloid precursor protein (APP) is most commonly known as the source of the β-amyloid (Aβ) peptides that accumulate in the brains of patients with AD. However, a large body of work supports the idea that APP is also a signaling receptor. Most recently, it has been shown that familial AD (FAD) mutations in APP or simple overexpression of wild type APP cause dysfunction of APP signaling, resulting in initiation of DNA synthesis in neurons and consequent apoptosis. In this article, we review the evidence that APP has the potential to activate aberrant neuronal cell cycle re-entry in AD, and we describe a signal transduction pathway that may mediate this abnormal activation of the cell cycle.

Keywords: Alzheimer's disease; Amyloid precursor protein; p21 activated kinase; APP–BP1; Cell cycle; Apoptosis

1. Introduction

AD is a progressive, incurable disease that always ends in death. Its predominant clinical manifestation is memory loss, but a number of other changes in brain function, including disoriented behavior and impairments in language, comprehension, and visual–spatial skills, also characterize this disorder. The diagnostic hallmarks of AD, namely neuronal attrition, amyloid deposits, and neurofibrillary tangles, have not as yet explained the etiology and pathogenesis of the disease. However, additional markers of AD have been described that may give some clues to the mechanism by which neurons die in AD brain. Notably, aberrant expression of cell cycle proteins and DNA tetraploidy in neurons in pathologically affected regions of AD brain have been described. It has been shown also that those neurons that have entered the cell cycle subsequently undergo a form of programmed cell called apoptosis. By now, the evidence that neuronal death in AD may be at least partly due to cell cycle entry followed by apoptosis, is considerable. This has clear therapeutic implications: understanding the molecular pathways underlying this cell cycle-mediated neurodegeneration will reveal new therapeutic targets and lead to novel strategies for slowing or even blocking the onset and progression of AD. One such pathway, that is mediated by APP, will be described.

2. Cell cycle abnormalities in AD

2.1. The cell cycle is activated in neurons in AD brain

A decade ago, Vincent and Davies showed definitively that activation of cell cycle components occurred in AD brain [1]. Subsequently, abnormal expression of such cell cycle molecules as cdc2, cdk4, p16, Ki-67, cyclin B1, and cyclin D was reported in pathologically affected or vulnerable neurons in AD brain [2–6]. Busser et al. [6] found ectopic expression of cell cycle proteins in regions of AD brain displaying extensive cell death, and Chow et al. [7] detected increased expression of genes

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encoding cell cycle proteins in single neurons in late-stage relative to early-stage AD brain. A number of these cell cycle regulators have been reported to appear in vulnerable neurons prior to lesion formation [6,8,9].

These data showed correlations between the expression of cell cycle markers and AD neuropathology, but did not reveal the consequences for AD neurodegeneration of such activation of cell cycle proteins. Yang et al. [10] showed that one of these consequences appears to be that vulnerable neurons in AD brain re-enter the cell cycle. This report demonstrated that a significant number of hippocampal pyramidal (4% vs. 0% in control hippocampus) and basal forebrain neurons in AD brain had undergone full or partial DNA replication, evidence that these neurons had completed the S phase of the cell cycle. Such DNA synthesis is not normally seen in neurons; and indeed, it was not detected in regions of AD brain untouched by pathology or in control brains. Yang et al. [10] suggested that the state of tetraploidy eventually is lethal to neurons. In a follow-up report, this same group [11] described the emergence of cell cycle-related proteins prior to neuronal cell death not only in the brains of individuals with advanced AD, but also in the brains of individuals with mild cognitive impairment, which usually progresses to AD. Yang and co-authors went on to describe the reexpression of cell cycle markers in four different plaque-bearing mouse models of AD [12]. In one of these models, cell cycle events were detected many months before the first appearance of plaques. These in vivo observations supported reports showing that at least two *in vitro* models for AD neurodegeneration featured re-activation of cell cycle entry in neurons [13,14].

2.2. Cell cycle activation may link to tangles in AD

Activation of the cell cycle may also contribute to the formation of neurofibrillary tangles, which are pathological markers for AD that contain hyperphosphorylated microtubule associated protein tau. The phosphoepitope S214 of tau, that appears in neurofibrillary tangles in AD, is a prominent phosphorylation site in metaphase but not in interphase of dividing cells expressing tau [15]. The tau TG3 epitope (phosphorylated T231) has been detected in mitotic cells but not in quiescent cells [1]. Moreover, tau is hyperphosphorylated during mitosis in neuroblastoma cells [16], and a cdc2-related kinase is associated with paired helical filaments, the ultrastructural correlates of neurofibrillary tangles, in AD brain [17]. Patrick et al. (1999) showed that activation of the p25/Cdk5 complex results in hyperphosphorylation of tau and reduces the ability of tau to associate with microtubules. Mitotic phosphorylation of tau likely causes conformational changes, which may be associated with the development of neurofibrillary tangles. These data support the idea that reactivation of the cell cycle machinery not only causes DNA synthesis but also may cause tau hyperphosphorylation in AD brain.

2.3. Cell cycle activation may lead to apoptosis in AD brain

Activation of cell cycle proteins in neurons also can, under certain circumstances, lead to a form of cell suicide called apoptosis (reviewed in ref. [18]). The possibility that such a process occurs in AD brain was first suggested by Su et al. [19], when they reported evidence for DNA fragmentation in neurons in AD brain. This same group showed that β-amyloid induces neuronal apoptosis in vitro [20], a finding that has been replicated in many laboratories. In support of the idea that apoptosis may be one means by which neurons die in AD, several groups have reported the presence of activated caspases in the postmortem brains of individuals with AD [21–23], and have described apoptotic neurons in the brains of transgenic mouse models for AD [24–27]. Apoptosis normally proceeds to completion within 16–24 h, which seems incompatible with the relatively slow progression of AD. However, Cotman [28] and Raina et al. [23] have both proposed that the induction of compensatory responses to apoptosis in the AD brain protects the neurons from terminal apoptosis, and that a dynamic balance between cell death processes and compensatory responses exists in AD brain.

3. APP signaling pathways that mediate cell cycle activation

3.1. APP is a signaling protein

The amyloid precursor protein (APP) is the source of the β-amyloid peptides that accumulate in the brains of patients with Alzheimer's disease (AD). The primacy of APP in the etiology of AD is underscored by the fact that virtually all individuals with Down syndrome (DS), who as a consequence of chromosome 21 trisomy overexpress APP, develop AD by the age of 40. This finding, together with the observation that either specific mutations in APP or simple duplication of the wild type APP gene can cause some forms of FAD [29,30], suggests the possibility that an alteration in the normal function of APP may occur in AD, and has refocused attention on the delineation of the function that APP subserves in the brain.

The notion that APP may be a signaling receptor was originally proposed on the basis of the predicted amino acid sequence of APP, which suggested that APP was a type 1 intrinsic membrane protein whose structure was consistent with that of a "cell surface receptor" [31]. The first direct evidence in support of this idea was the finding that the APP cytodomain interacted with and activated the heterotrimeric G protein G_o [32], a finding that was subsequently confirmed independently [33]. During the last decade, a multitude of additional cytosolic proteins that interact with the APP cytodomain have been described [14,34–43], suggesting that APP has versatile signaling roles.

To define neuronal signaling pathways that may be mediated by APP, we established an in vitro paradigm for AD neurodegeneration, in which wild type or FAD APP cDNAs were expressed as transgenes in primary cortical neurons [14,44]. Using this model, we showed that overexpression of wild type or even modest expression of FAD mutants of APP in neurons leads to neuronal DNA synthesis and apoptosis [14,44], suggesting that an APP-mediated signaling pathway may play a role in the cell cycle activation and neurodegeneration seen in AD.

3.2. APP–BP1, which binds to APP, is a cell cycle protein

As a first step in defining neuronal signaling pathways that may be initiated by APP, a search of brain cDNA libraries was carried out to detect genes encoding proteins that interact with the cytodomain of APP [14,43]. One of these, APP–BP1, was found to be an essential cell cycle protein that drives the cell cycle through the S-M checkpoint in dividing cells, but which causes apoptosis when overexpressed in neurons [44]. Overexpression of wild type APP or expression of FAD mutants of APP in neurons, using the paradigm described above, resulted in an increase in expression of APP–BP1 in lipid rafts, followed by entry of the neurons into the S phase of the cell cycle, and neuronal apoptosis [44]. As predicted by these in vitro results, APP–BP1 was shown to be overexpressed in lipid rafts in at-risk regions of human AD brain relative to cognitively intact controls [45]. Consistent with these observations, modest overexpression of APP–BP1 in neurons, sufficient to mimic the observed increases of this protein in lipid rafts in AD brains, caused neuronal cell cycle entry and apoptosis [45].

The interaction of APP with APP–BP1 activates a pathway leading to the conjugation of NEDD8, a ubiquitin-like protein, to its target (Fig. 1, ref. [44]). APP–BP1, together with hUba3, is functionally analogous to the ubiquitin activating enzyme E1, with hUba3 containing the active cysteine and ATP binding site. When NEDD8 is activated by the APP–BP1/hUba3 complex, it forms a thiol ester bond with hUbc12, which has a function parallel to that of ubiquitin-conjugating enzyme Cdc32. Subsequently, NEDD8 is covalently coupled to lysine residues in its target proteins [46]. So far, the proteins known to be neddylated via this pathway are a family of proteins called cullins [47] and the Mdm2 oncogene product, which in turn regulates neddylation of the cell cycle protein p53 [48]. Cullins are scaffold proteins for the E3 ubiquitin ligase complex, and neddylation of cullin enhances its ability to promote ubiquitination [49,50]. Indeed, NEDD8 has been found in ubiquitinated neurofibrillary tangles in AD brain [51]. NEDD8 signalling has been shown to regulate protein degradation pathways participating in cell cycle progression [52–55]. The discovery of a novel protein, NUB1, which recruits NEDD8-conjugates to the proteasome for degradation, provides a direct link between these two systems [56,57]. Inhibition of the neddylation pathway in neurons by expression of a dominant negative mutant of hUbc12 prevents FAD APP-mediated cell cycle entry and apoptosis [44,45]. Thus, elements of this pathway are attractive targets for potential therapies aimed at preventing neurons in AD brain from entering the cell cycle.

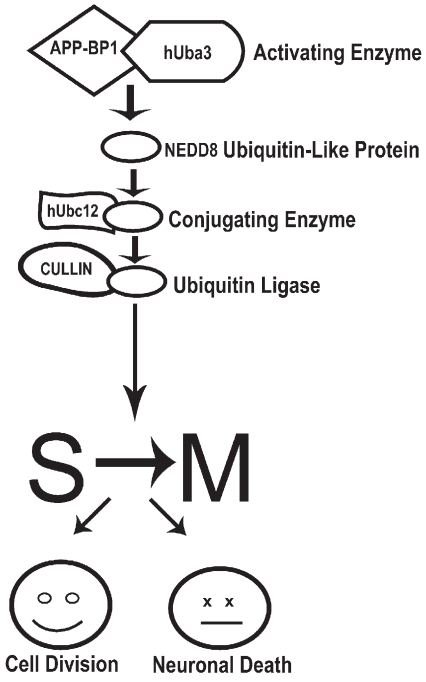

Fig. 1. Graphic representation of the neddylation pathway activated by the interaction of APP with APP–BP1. Steps in the cascade that are potential therapeutic targets include the interaction between APP and APP–BP1, and the interaction of APP–BP1/hUba3 with the conjugating enzyme hUbc12.

3.3. p21 activated kinase 3 mediates neuronal DNA synthesis caused by FAD mutants of APP

In addition to APP–BP1, the p21-activated kinase PAK3 also was shown to bind to the C-terminus of APP [14]. PAK3 is a serine/threonine kinase whose activity is regulated by the p21 family of small GTPases. Mutations in this molecule, which is expressed selectively in the nervous system [14], cause one form of X chromosome-linked mental retardation [58,59]. PAK3 is involved in the control of cytoskeleton dynamics, possibly affecting cognition by regulating the shape of neuronal synapses. Consistent with this idea, mice lacking the *Pak3* gene are impaired in both synaptic plasticity and cognition [60].

PAK3 also has been implicated in the abnormal DNA synthesis and apoptosis caused by overexpression of wild type APP or ectopic expression of FAD mutants of APP in neurons in vitro [14]. A graphic representation of the neuronal signaling pathway activated by interaction of FAD APP with PAK3 is shown in Fig. 2. A dominant negative kinase-dead mutant of PAK3 inhibits wild type or FAD APP-mediated neuronal apoptosis and DNA synthesis in our in vitro model; this effect is abolished by deletion of the PAK3 APP-binding domain or by co-expression of a peptide representing this binding domain [14]. These data suggest that both the kinase activity of PAK3 and also its interaction with APP are important for the FAD APP signaling pathway. The specificity of PAK3 involvement in FAD APP-mediated apoptosis rather than in general apoptotic pathways is suggested by the facts that a constitutively active mutant of PAK3 does not alone cause neuronal apoptosis, and that the dominant negative mutant of PAK3 does not inhibit chemically-induced apoptosis. In vitro, FAD APP-mediated DNA synthesis precedes FAD APP-mediated apoptosis in neurons; and inhibition of neuronal entry into the cell cycle blocks the apoptosis [14].

Pertussis toxin inhibits DNA synthesis and apoptosis caused in vitro by FAD APP mutants, implicating G_o in the process as well [14]. These data are consistent with the reports by Nishimoto and colleagues that the His^{657}–Lys^{676} domain of

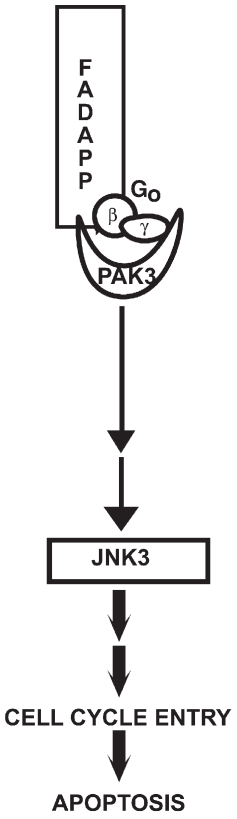

Fig. 2. Graphic representation of the signaling pathway activated by the interaction of PAK3 with APP and G_o. Steps in the cascade that are potential therapeutic targets include interactions among APP, G_o and PAK3 or the activity of JNK3.

APP-695 activates the heterotrimeric GTP-binding protein G_o in a $GTP_\gamma S$-inhibitable manner [32,61]. Their demonstration that an antibody to the extracellular domain of APP that acts as a ligand mimetic [62] causes activation of G_o, argues that APP may be a G protein-coupled receptor. Notably, neuronal expression of FAD APP deleted for residues His^{657}–Lys^{676} does not cause cell cycle entry [14].

These data support a model in which APP normally is part of a G_o protein-centered complex including PAK3 that transduces extracellular signals to the cytoplasm (Fig. 2), and in which overexpression of wild type APP or expression of FAD APP causes dysfunction of this pathway. How might this complex be related to the pathway activated by APP–BP1? At least one of the downstream molecules in the PAK3-mediated pathway is the c-Jun N-terminal kinase 3 (JNK3). A dominant negative mutant of JNK3 inhibits FAD APP-mediated cell cycle entry, while a constitutively active mutant of JNK3 alone causes neuronal DNA synthesis (McPhie, D.L. and Neve, R.L., unpublished data). The effect of the constitutively active JNK3 is not blocked by a dominant negative mutant of PAK3, implying that the action of JNK3 is downstream of PAK3. However, inhibition of the APP–BP1-mediated neddylation pathway in neurons by expression of a dominant negative mutant of hUbc12 prevents constitutively active JNK3 activation of neuronal cell cycle entry (Chen, Y. and Neve, R.L., unpublished data). Whereas pertussis toxin blocks neuronal DNA synthesis caused by ectopic expression of FAD APP, it does not block neuronal DNA synthesis caused by APP–BP1. These data suggest that the order of events in the FAD APP-mediated pathway leading to neuronal cell cycle entry and apoptosis consists of formation of the APP-G_o–PAK3 complex, followed by activation of JNK3, which is then followed by activation of the APP–BP1 neddylation pathway. Intermediate events are likely to occur.

These findings also implicate JNK3 in FAD APP-mediated neuronal DNA synthesis and apoptosis, and are consistent with the finding that JNK3 is highly expressed and activated in postmortem brains of individuals with AD [63]. JNK3 is associated with neurofibrillary tangles, and JNK upregulation colocalizes with phosphorylated tau [63], a microtubule associated protein which has been shown to be phosphorylated by JNK [64]. Abnormal phosphorylation of tau by JNK3 causes the formation of oligomeric tau fibrils that have been termed "pretangles" [65]. In a transgenic mouse model of AD, JNK activation is associated with amyloid deposits and phospho-tau. Age-dependent increased JNK activity is correlated not only with increased amyloid deposition in this mouse model, but also loss of functional synapses similar to that observed in AD brain [66].

We and others have hypothesized that APP and its C-terminal binding proteins – among them G_o, PAK3, and APP–BP1 – have a regulated interaction that activates signaling pathways important for normal brain function, perhaps mediating synaptic remodeling or neurogenesis during learning. In analogy with the cell proliferation and resultant tumorigenesis caused either by a constitutively active mutant of the epidermal growth factor receptor (EGFR) or by sustained overexpression of wild type EGFR (Lorimer, 2002), neuronal cell cycle entry and consequent apoptosis may be caused either by constitutive activation of APP signaling pathways in neurons due to FAD mutations in APP or by sustained overexpression of wild type APP. Thus, therapeutic reagents that target these signaling pathways may have efficacy in treating AD neurodegeneration.

3.4. APP-C31 may mediate neuronal cell cycle entry and neurodegeneration in AD brain

All but one of the binding proteins for the APP cytodomain interact with APP within the last 31 amino acids of this domain. Why is this important? For one thing, C31 can be generated from APP by caspase cleavage [67,68]. Furthermore, this cleavage has functional significance, in that expression of C31 alone has been shown to cause neuronal DNA synthesis and apoptosis [14,45,68]. Recently, inhibition of C31-producing caspase cleavage of APP was shown to prevent the development of AD-like pathology and behavior caused by two FAD mutations of APP [69]. A D664A mutation (which prevents the generation of C31) was introduced into the background of a human APP minigene carrying the K670N/M671L (Swedish) and V717F (Indiana) mutations. Both the original FAD mutant minigene (PDAPP) and also the D664A version of it [PDAPP (D664A)] were expressed in transgenic mice under the control of the PDGF B-chain promoter.

Interestingly, the authors showed that the D664A mutation neither altered the net *in vivo* production of Aβ40 and Aβ42, nor affected the extent of amyloid plaque deposition in the brains of PDAPP(D664A) mice compared to PDAPP mice. However, the D664A mutation *did* have an effect on neurodegeneration and on behavior. The PDAPP mice without the D664A mutation displayed decreased hippocampal pre-synaptic density number relative to controls at 8–10 months of age, a pronounced increase in GFAP immunoreactivity in the hippocampus by 12 months, a loss in dentate gyrus volume at 3 months, learning and spatial impairments at 12 months, and an increase in the number of proliferating cells present in the subgranular zone of the dentate gyrus at both 3 and 12 months. In contrast, the PDAPP(D664A) mice, with the D664A mutation, were indistinguishable from controls in every one of these parameters.

It can be inferred from these data that the mutation Asp664 "rescues" multiple aspects of neuropathology and impaired learning that are normally caused by the Swedish and Indiana mutations in APP. In other words, if C31 cannot be generated, the FAD APP mutations cannot cause certain pathological and behavioral abnormalities. What are the implications of these findings? First of all, note that Asp664 selectively rescues the neurodegeneration and the learning abnormalities of the PDAPP mice without decreasing the production of Aβ40 or Aβ42. Thus, the rescue is independent of the production of Aβ. Secondly, the C31 region of APP encompasses the binding sites for nearly all of the signaling proteins, including APP–BP1 and PAK3, that have been shown to bind to the intracellular domain of APP.

The data suggest a scenario in which C31, when removed from APP, abnormally activates or disrupts signaling pathways mediated by APP. One possibility is that the signaling initiated by interaction of the APP C-terminal domain with signal transduction proteins normally is tightly regulated by binding of ligand(s) to its extracellular domain [variously identified as F-spondin [70], notch [71,72], TGF-β2 [73], and even Aβ [74,75]. In such a scenario, as suggested by McPhie et al. [14], C31, when not attached to APP, is relieved of the normal constraints imposed on it when the extracellular domain of APP is not occupied by a ligand, and becomes constitutively active or else takes on signaling functions that it does not normally possess (Fig. 3).

To test the idea that C31 becomes constitutively active, one may ask whether causing APP signaling to become tonically active results in the same consequences for the neuron that expression of C31 alone does. It has been found that exposure of neuronal cells to the antibody 22C11, raised against the extracellular domain of APP, causes constitutive activation of G_o [62] which is known to bind to and be activated by APP [32,33]. In this context, 22C11 could be considered to be an APP ligand mimetic of sorts. Indeed, sustained exposure of neurons to 22C11 causes DNA synthesis [14] and neuronal apoptosis [14,76,77] via the APP–BP1- and PAK3- mediated signal transduction pathway that is activated by C31 alone [14].

4. Concluding remarks

We have proposed that, in neurons, APP is a signaling receptor whose normal function is tightly regulated by ligand binding (Fig. 3). We hypothesize that this normal, regulated function is altered by overexpression of wild type APP or by expression of FAD mutants of APP, leading to neuronal cell cycle entry and consequent apoptosis. We have reported previously that, in contrast to FAD mutant APPs, neither wild

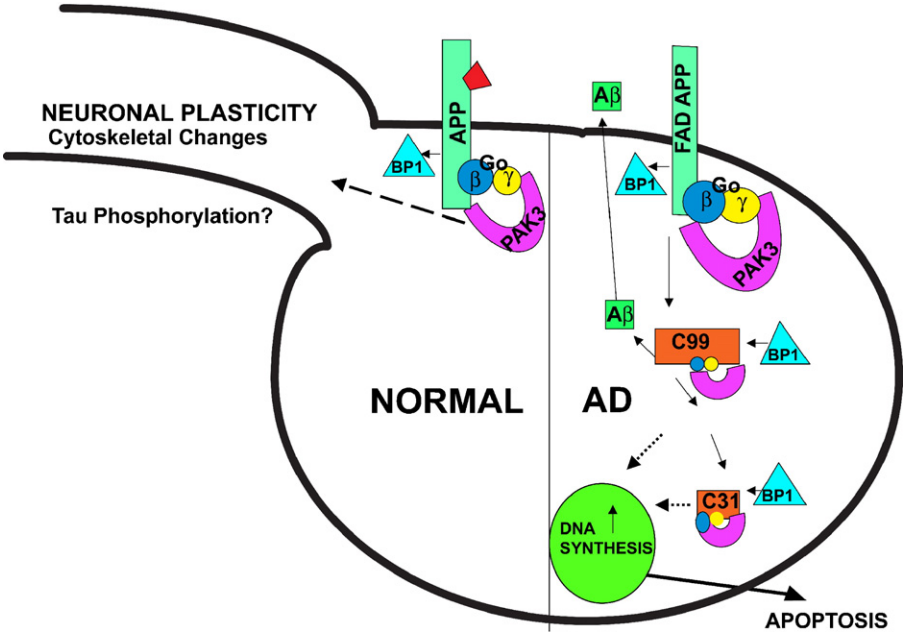

Fig. 3. Schematic of a signal transduction pathway activated by FAD mutations of APP. In the normal cell, the function of APP is tightly regulated by the binding of its ligand (portrayed in the figure as a trapezoid). FAD APP is relieved of such constraints, and, together with PAK3, G_o, and APP–BP1, causes aberrant neuronal DNA synthesis and apoptosis.

type nor FAD mutant presenilin enhance apoptosis in neurons [78]. In addition, we have shown (McPhie, D.L. and Neve, R.L., unpublished data) that neither wild type nor FAD mutant presenilin causes aberrant neuronal cell cycle entry. It is worth noting that expression of FAD APP leads to increased levels of secreted α-APP and β-APP, and to elevation of levels of specific C-terminal fragments of APP in addition to Aβ, while the only known effect of FAD presenilins on APP processing is alteration of the ratio of Aβ [42] to Aβ [40]. These data suggest that fragments of APP other than Aβ may play a role in abnormal neuronal cell cycle entry and consequent apoptosis, and also suggest that there is more than one pathway to neurodegeneration in AD.

These data highlight the importance of understanding the normal function of APP in the brain and how this function may be disturbed in AD.

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